

MAPPER: A Software Program for Quantitative Biofilm Characterization

C.R. Tolle*, T.R. McJunkin, and D.L. Stoner

Idaho National Engineering and Environmental Laboratory

P. O. Box 1625

Idaho Falls, ID 83415-2210

Abstract

Biofilms are complex, structurally heterogeneous microbial communities. The reliance on verbal or visual description methods has limited the ability to predict and understand the mechanisms of biofilm formation. For that reason, increasing attention is being paid to quantify verbal and visual descriptions into numerical quantities so that empirical models might be developed that both track as well as predict biofilm growth in real time. Numerical models will be compared and contrasted with analytical models and used to: 1) elucidate the mechanisms of biofilm formation; 2) investigate the dynamics of these complex systems; and 3) provide a conceptual framework for experimentation. To this end, a software program entitled MAPPER was developed to generate a topographical mapping over sub-regions of a biofilm via fractal dimension and lacunarity quantification. Biofilm growth characteristics can be tracked by analyzing changes in the topographical maps. In order to speed the quantification process, the software program was designed for a distributed architecture using multiple hardware and system configurations via a secure network protocol. We achieve near one to one scaling of processing speed increase of a biofilm image with each additional node added to a mixed network of x86 Linux, Alpha Linux, and MacOS X computers running OpenSSH. No special network or hardware is required such as a Beowulf (we can, however, take advantage of Beowulf hardware) computer. A graphical user interface (GUI) was developed to facilitate interaction among scientists from the key disciplines of microbiology and applied mathematics. The software's GUI was implemented in TCL/Tk and is portable to all major operating systems that have an implementation of TCL/Tk, OpenSSH, and GNU gcc (this includes Linux, Unix, MacOS X, and Windows). The software program was developed as a diagnostic tool for assessing the mechanisms of biofilm structure and has been evaluated using microscopic biofilms and synthetic biofilm images.

Quantifying biofilms.

A signal or image can be measured using a variety of statistics. One statistic that has value and is commonly used is fractal dimension. In many cases fractal dimension provides an incomplete description of the texture (i.e. two images can have same fractal dimension but look markedly different). Another statistic mentioned occasionally is lacunarity. This has been employed as a second order statistic. We have several new ways to measure lacunarity that go to the Latin root of the word--Lacuna meaning gap. These overcome problems associated with the common lacunarity measure, glide box. Refer to the back side of this presentation for more details on fractal dimension and lacunarity definitions.

"Mapper" is a TCL/Tk user interface built for controlling the segmenting and processing of images over a distributed cluster of computers. The user has control of how the image is segmented and defines any necessary parameters for the processing program. The program copies all necessary scripts and binaries to each of the nodes using the preferred secure shell (ssh) or remote shell (rsh).

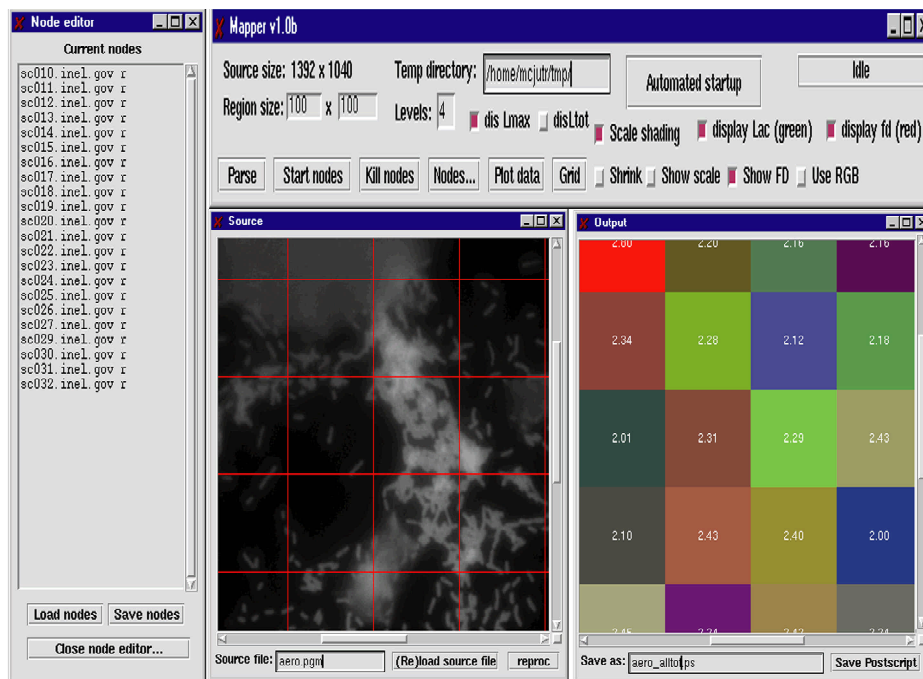
Mapper launches a client script on each node which waits for an assignment. Mapper creates a file for each node with the filename of the image segment assignment. When done processing the segment the client copies the results back to the host node and receives another assignment if there are remaining segments. Mapper then displays the results in the interface.

This remarkably efficient method of achieving massive parallelism in processing large images, provides near one to one scaling with each additional processor.

"Mapping" a biofilm

In a biofilm, like the one shown and analyzed in this presentation, we would like to characterize the growth behavior of various regions. The approach taken here is to divide the image of the biofilm up into rectangular regions and characterize each region with fractal dimension and lacunarity parameters. This is useful as an analysis tool if the behavior of a region can be related to this set of statistics.

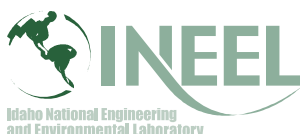
The parameters are represented in a composite of Red (for fractal dimension), Green (for lacunarity Musgrave parameter), Blue (for either lacunarity maximum of the minimum spanning tree, or the total span of that tree). The result of this mapping is shown in the composite RGB image overlaid on the biofilm. The color map shows these parameters taken in concert to show contrasts in the types of biofilm growth patterns.



Mapper graphical user interface

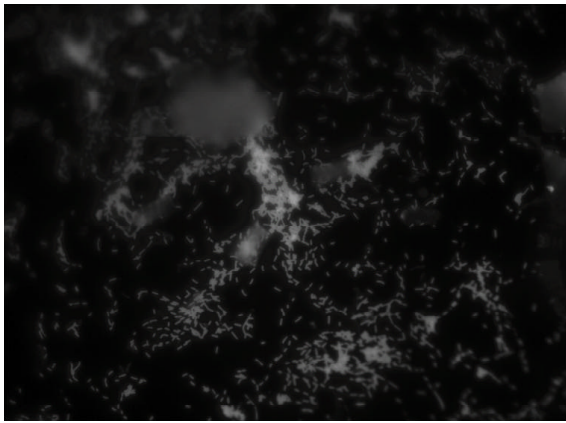
*Corresponding author
e-mail: tollcr@inel.gov
voice: (208) 526-1895
FAX: (208) 526-0690

P. O. Box 1625
Idaho Falls, ID 83415-2210

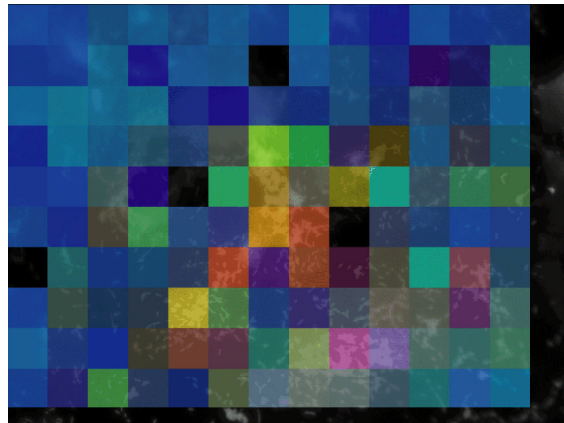


A science-based, applied engineering national laboratory dedicated to supporting the U.S. Department of Energy's missions in environment, energy, science and national defense. It is operated for DOE by Bechtel BWXT Idaho, LLC under contract DE-AC07-99ID13727





Biofilm image



Composite overlay of fractal dimension and lacunarity on a biofilm image.

Fractal Dimension

Fractal Dimension (FD) is a measure of how much space is filled in a signal or image. The simplest example of a set with a specific fractal dimension is the Cantor dust, where each subsegment is made up of a scaled copy of the previous scale. The diagram at the right shows the construction of a Cantor set where the scaling factor is 3 and 2 copies of each scale are made at each successive level. $FD = \text{Log}(2)/\text{Log}(3)$ using the similarity dimension.

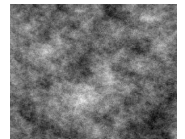
Our highly accurate estimator of FD for images uses fuzzy-c means clustering to find the slope of the log of the size of the cluster vs. the log of the number of clusters, e.g. an approximation the Hausdorff FD measure. Images to the right show textures on which our algorithm was validated.



Cantor Dust Construction



FD = 2.1



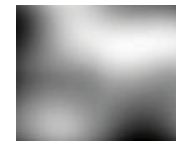
FD = 2.7

Brownian Random Fractal Textures

Lacunarity (Dense/Image Data)

Musgrave's Brownian random fractal texture generating algorithm shows that there is another parameter which can effect the look of a texture. To the right are 3 more textures (limited to 2 self similar scales) with various Lacunarity parameters (LP).

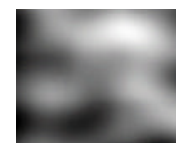
We developed another algorithm that uses the same clustering methods as our FD estimator for approximating Musgrave's parameter. This method creates a histogram of the length of line segments connecting two cluster which do not pierce any other cluster. The reciprocal of the midpoint of the steepest slope gives us the parameter. The graph at the right shows an example of the histogram and parameter estimation.



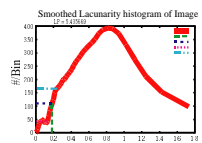
LP = 2



LP = 5

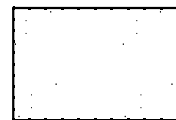


LP = 3



Lacunarity (Max Span - Lmax)

Ramified data sets can also be quantified with Fractal Dimension measures; here again, two sets with similar FD can have very different forms. To the right are 2D Cantor Dusts which have the same FD but a different look. Lmax and Ltot Lacunarity measures have the capability to distinguish between these sets. The graphics below to the right show the formation of the minimum spanning tree. Lmax is the length of the longest span in this tree. This measure was not originally thought to be useful in analyzing images; however, it does appear to help distinguish between different types of growth in some cases.



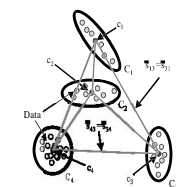
2D Cantor Dusts fixed FD with differing Lacunarities

Lacunarity (Total Span - Ltot)

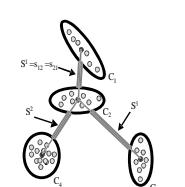
Ltot is the sum of the lengths of the segments of the minimum spanning tree. This measure, although also designed for ramified data has been somewhat useful with biofilm images.

References

1. C.R. Tolle, T.R. McJunkin, D.J. Gorsich, *Sub-optimal minimum cluster volume cover based method for measuring fractal dimension*, submitted for review to IEEE Pattern Recognition Analysis and Machine Intelligence (in 2nd review 2002).
2. C.R. Tolle, T.R. McJunkin, D.T. Rohrbaugh, R.A. Laviolette, *Optimal-cover based definitions of lacunarity for ramified data sets*, submitted for review to Physica-D 2002.



Clusters and all spanning segments



Minimum spanning tree